Journal of Radioanalytical and Nuclear Chemistry
https://doi.org/10.1007/s10967-019-06746-9

Neutron activation analysis as a tool for tracing the accumulation of silver nanoparticles in tissues of female mice and their offspring

Inga Zinicovscaia[1,2] · Dmitrii Grozdov[1] · Nikita Yushin[1] · Alexandra Ivlieva[3] · Elena Petritskaya[3] · Dmitriy Rogatkin[3]

Received: 23 July 2019

Abstract
The silver accumulation in different tissues of female mice and their offspring after prolonged oral administration of silver nanoparticles to the females during pregnancy and lactation was investigated. Silver content in different organs (blood, liver, brain, kidney and lungs) was determined by means of neutron activation analysis. According to the obtained data silver nanoparticles are able to reach and cross the placental barrier and blood-to-brain barrier in both mice female and their offspring. In mice female the highest silver concentration was determined in lungs, followed by brain, liver, kidney and blood. In offspring silver bioaccumulation changed in the following order lungs > brain > blood > liver > kidney. The average specific mass content of silver which crossed the blood–brain barrier was 373 ± 75 ng (for female) and 385 ± 57 ng (for offspring). The obtained results are important for studies in developmental and reproductive toxicity of nanomaterials.

Keywords Silver nanoparticles · Brain · Liver · Lungs · Kidney · Blood · Mice · Offspring · Distribution · Neutron activation analysis

Introduction

With increasing production and application of nanomaterials in industry and medicine, the importance of assessing the bioaccumulation characteristics and potential risks of nanomaterials to human health is growing [1]. Recently, interest in the potential impact of consumer-relevant engineered nanoparticles on pregnancy and developing fetuses has dramatically increased [2]. Several biological barriers prevent uncontrollable exposure of tissues to substances, including potentially harmful, carried by blood: blood–brain, blood–testis in males or blood–follicle in females and the blood–placenta barrier, which controls exchange of substances between fetus and its mother [3]. In a female organism silver nanoparticles (AgNPs) can also be transferred to breast milk by means of transcytotic, membrane transport, and paracellular transport pathways [4].

In recent years, the number of studies investigating transport of nanomaterials through the blood–placenta barrier and also through milk during the breast-feeding and their effects on the fetuses and newborns is significantly increasing. However, once inside the body nanoparticles are distributed to different organs by the blood flow, especially to the ones that contain large numbers of phagocytic cells (liver, spleen, lung) [5], as well as in the brain—passing through the blood–brain barrier.

Metal nanoparticles distribution in different organs depends on administration route, particles size, type of coating [6–8]. Gold nanoparticles of 18 nm size were retained nearly completely in lungs, whereas the 1.4-nm size nanoparticles were found in significant amounts in blood, liver, skin, and carcass [9]. The silica (70 nm) and titanium oxide (143 nm) nanoparticles were able to cross the placental barrier and accumulate in the liver and brain of fetuses. However, nanoparticles of a greater size, 300 nm or 1000 nm, were not observed in placenta or fetuses [8]. Iron nanoparticles possessing a negative surface potential was observed to have highest accumulation in livers and spleens, while nanoparticles from positively charged polyethylene glycol and polyethylenimine (PEG-PEI) coated nanoparticles was

✉ Inga Zinicovscaia
zinikovskaia@mail.ru

[1] Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russian Federation 141980

[2] Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, Bucharest, Magurele, Romania

[3] M.F. Vladimirskiy Moscow Regional Research and Clinical Institute, Moscow, Russian Federation 129110

Published online: 03 September 2019

observed to have the highest concentration in lungs [10]. Yang et al. [1] investigated the distribution of gold nanoparticles with different coating in organisms of pregnant mice and their offspring. Particles with a negative surface coating exhibited reduced uptake in fetus, while PEG and ferritin-coated nanoparticles showed a high rate of accumulation in female organs and offspring.

The nanoparticles' distribution also depends on the stage of pregnancy at which nanoparticles were administrated. Sweeney et al. [6] showed that mesoporous silica nanoparticles administrated to females during the early gestation were able to pass placental barrier and accumulate in the fetuses, while in case of administration during the late gestation the permeability of placental barrier for silica nanoparticles had significantly decreased. Gold nanoparticles administered late in pregnancy had almost no transfer from mother to fetus [11].

The uptake of metal nanoparticles in tissues was traced using several analytical techniques: laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), sector-field-ICP-MS (SF-ICP-MS) [12], inductive coupled plasma atomic emission spectrometry (ICP-AES) [7], single-particle inductively coupled plasma mass spectrometry, transmission electron microscopy coupled with energy-dispersive X-ray spectroscopy [2], inductively coupled plasma mass spectrometry (ICP-MS) [1, 13], atomic absorption spectrometry (AAS) [1].

The application of above mentioned techniques is explained by the possibility of multi-elemental analysis, high selectivity, sensitivity and low detection limits for most of the elements [14]. At the same time, they are characterized by difficult sample preparation procedure and decrease of the sensitivity at low concentration of elements in tissue samples [15]. Neutron activation analysis due to its non-destructive character proved to be an efficient technique, which allows determination of a wide range of elements with high precision in small samples [16].

The aim of the present study was to examine the distribution of silver nanoparticles in different tissues of female mice and their offspring in comparison to control animals after oral exposure of experimental females to silver nanoparticles during pregnancy and lactation. Neutron activation analysis was used to determine total silver content in blood, brain, kidney, liver, and lungs samples.

Materials and methods

Silver nanoparticles

Polyvinylpyrrolidone coated silver nanoparticles (AgNPs) with the average diameter 8.7 ± 1.4 nm were purchased in form of concentrated (13 mg/ml) colloid solution "Argovit-C" from SPC *Vector-Vita* (Novosibirsk). The experimental solution of concentration 25 μg/ml for consumption by mice was prepared by dilution of concentrated solution with pure water in a ratio of 1:500.

Animals

Outbred white mice (SHK) of the age 1.5–2 months (with an average mass of 20 g), 30 females and 6 male, were purchased from the Stolbovaya Farm (Moscow region, Russia). Mice are heterozygous by undefined number of genes and are used to assess the safety of medical and cosmetic products, and dietary supplements. The animals and their offspring were maintained in the vivarium of M.F. Vladimirskiy Moscow Regional Research and Clinical Institute. Females were kept in steel cages with sizes of $31.5 \times 23 \times 15.7$ cm, and each cage contained 5 mice, with a natural cycle of illumination and the temperature of 22–24 °C; each cage was cleaned once per day. The methodology of the experiments and the maintenance of the animals at the vivarium of the institute were performed according to the principles of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Experimental design

To study uptake of AgNPs both for mothers and, by proxy, the offspring experimental females (15 individuals) were drinking the AgNPs solution with concentration of 25 μg/ml since one week before pregnancy and to the end of lactation (1 month after birth). Control females (15 individuals) drank pure water all the time. Thus, the offspring of experimental females received AgNPs from mothers, through the placental barrier during prenatal development and with milk during lactation. At the same time the offspring of control females had no contact with AgNPs.

At the end of the lactation period females and a part of offspring were euthanized. In experimental biology, medicine and veterinary there are several methods of euthanasia: hypoxia (direct or indirect), use of injectant and drugs overdose. Use of the injectable drug is considered an optimal (quick and safe) method of euthanasia, because it does not cause morbidity or fear in animals [17].

In the present work the euthanasia was performed by intraperitoneal injection of urethane solution. The concentration of the water solution based on body weight and dosage of 1.2 g of dry urethane per kg of body weight, proposed by a veterinarian. Brain, liver, lungs, kidneys and blood (with its volume measured) were extracted from each mouse, packed in aluminum foil (or plastic test tube in case of blood) and dried at a temperature of 75 °C until constant weight.

Neutron activation analysis

The content of silver accumulated in tissues was determined using neutron activation analysis at the IBR-2 reactor in Dubna, Russia. The description of irradiation channels and pneumatic transport system of the REGATA installation can be found in [16] and the procedure of tissues irradiation is described in [18].

Tissue samples were packed in aluminium foil cups and irradiated with epithermal neutrons in the irradiation channel with a cadmium shield during 3 days at a neutron flux of 1.8×10^{11} cm^{-2} s^{-1}. The samples were irradiated simultaneously with two reference materials: SRM 2710 (Montana Soil, Highly Elevated Trace Element Concentrations (NIST, USA) and 2891 (Cupric Sandstones, polymetallic ores, Karaganda, Kazakhstan (LLP «Centrgeolanalit»)) as well as with samples of packaging foil (blanks). The use of reference materials with the matrix different from the analyzed samples is explained by insignificant matrix effect in case of small samples (size and weight) [19].

Gamma spectra of induced activity were obtained using three spectrometers based on HPGe detectors with an efficiency of 40–55% and resolution of 1.8–2.0 keV for total-absorption peak 1332 keV of the isotope ^{60}Co and Canberra spectrometric electronics after 20 days for 1.5 h.

The analysis of the spectra was performed using the Genie2000 software from Canberra, with the verification of the peak fit in an interactive mode. The calculation of concentration was carried out using software "Concentration" developed in FLNP [20]. In the present study reference material SRM 2710 was used as a calibrator, and SRM 2891 was used to check the measurements accuracy. The obtained values for concentrations for SRM 2891 differed from the certified values in the range (2–10%).

Results and discussion

According to NAA data (Table 1) the mean content of silver in the blood was approximately four times higher in the offspring in than in females. The results are in agreement with [4] who showed better AgNPs absorption by the pups in comparison with the adults. In the case of oral administration, the higher accumulation of AgNPs in pups can be explained by higher intestinal permeability in the pups than in adults.

Also it should be noted that the amount of silver in the blood of the females after pregnancy and lactation was twice lower than data obtained in our previous study [18] when females were administrated with AgNPs for two-months, but without reproduction. Thus, it can be concluded that considerable quantities of AgNPs had been transferred to the offspring.

The liver, spleen, kidneys, and lungs are the major target organs which can be affected by nanoparticles. The highest content of silver was detected in the lungs, both for mice female and offspring (Table 2), that can be explained by a high amount of blood vessels in this organ, which can contribute to the increase of the content of silver accumulated by lungs. Campagnolo et al. [2] investigating the effect of AgNPs on the mice during the pregnancy also showed that nanoparticles had accumulated in all analyzed organs (lungs, liver, spleen, placenta), but with the highest accumulation in lungs.

The liver is the major distribution site in the body due to its high blood irrigation and the phagocytosis of NPs by Kupffer cells [21]. The Student's *t* test was applied to reveal differences between silver accumulation in liver of females and offspring. No significant differences ($p > 0.05$) between the obtained values (Table 3) were observed. The lower content of silver in the liver in comparison with the lungs can be explained by its excretion from the liver into the bile [5] or formation of silver–thiol complexes with their further excretion from the organism. High nanoparticles accumulation in liver was reported in several studies [1, 5, 7, 21, 22].

Table 1 Silver content in the blood of female mice and their offspring

Sample	Content (μg/g dry weight)			
	Female		Offspring	
	Range	Mean ± SD	Range	Mean ± SD
Control	0.21–0.26	0.23 ± 0.03	0.3–1.6	0.8 ± 0.5
Experimental	0.97–1.3	1.1 ± 0.1	2.6–5.8	3.9 ± 1.2

Table 2 Silver content in the lungs of female mice and their offspring

Sample	Content (μg/g dry weight)			
	Female		Offspring	
	Range	Mean ± SD	Range	Mean ± SD
Control	0.54–0.67	0.57 ± 0.09	0.5–2.0	1.1 ± 0.5
Experimental	5.4–8.6	6.7 ± 1.4	2.8–7.8	5.3 ± 1.7

Table 3 Silver content in the liver of female mice and their offspring

Sample	Content (μg/g dry weight)			
	Female		Offspring	
	Range	Mean ± SD	Range	Mean ± SD
Control	0.08–0.15	0.11 ± 0.03	0.1–0.4	0.28 ± 0.09
Experimental	3.2–5	3.9 ± 0.9	2.0–5.3	3.7 ± 1

Table 4 Silver content in the kidneys of female mice and their offspring

Sample	Content (μg/g dry weight)			
	Female		Offspring	
	Range	Mean ± SD	Range	Mean ± SD
Control	0.09–0.11	0.1 ± 0.09	0.5–2.3	1.7 ± 0.9
Experimental	1.6–3.5	2.5 ± 0.8	1.6–3.8	2.9 ± 1.2

The amount of silver accumulated in the kidneys of females and offspring was lower in comparison with its content in lungs, liver and brain samples (Table 4), which can be explained by kidneys' capacity for rapid elimination of toxicants from the vascular compartment during blood filtration. Thus, kidneys play a key role in the transport and clearance of nanoparticles in vivo [22, 23]. In case of kidneys, according to Student's *t* test statistically significant differences ($p < 0.05$) for values obtained for experimental mice and offspring were observed.

The results presented in Table 5 showed that almost the same amount of silver was accumulated by females' and offspring's brain. Silver content in experimental animals was much higher than in control ones, indicating penetration of AgNPs through the blood–brain barrier and accumulation in the brain. Since AgNPs were administrated in female before and during pregnancy, it is suggested that a part of them was able to reach the brain before the blood–brain barrier was formed in the fetus. Takeda et al. [3] found that TiO_2 nanoparticles administrated subcutaneously to pregnant mice were transferred from the mother to the fetal brain.

The amount of silver accumulated in brain of females and offspring was higher than in liver and kidneys, which is in agreement with Morishit et al. [4] research.

The brain receives blood from two sources: the internal carotid arteries and the vertebral arteries [24]. Thus, the silver content in the brain samples consists of the accumulated silver and the silver from the inside of the blood capillaries which were caught by during the sample preparation. To calculate the specific mass content of silver, which crossed the blood–brain barrier and thus accumulated in brain, by excluding the silver contained in blood vessels, the methodology described in Antsiferova et al. [25] was applied. In calculations additional information about specific activity of the blood with respect to ^{110m}Ag and ^{59}Fe (not shown here) was used and the obtained values are presented in Table 6.

Table 5 Silver content in the brain of female mice and their offspring (including silver in blood vessels)

Sample	Content (μg/g dry weight)			
	Female		Offspring	
	Range	Mean ± SD	Range	Mean ± SD
Control	0.3–0.9	0.5 ± 0.3	0.3–2.5	1.2 ± 0.9
Experimental	4.1–5.1	4.4 ± 0.4	3.2–5.5	4.8 ± 0.5

Table 6 Specific mass of silver accumulated in the brain of female mice and their offspring, ng

Sample	Female		Offspring	
	Range	Mean ± SD	Range	Mean ± SD
Control (2 months)	14–20	17 ± 4	11–36	16 ± 5
2 months of administration	305–477	373 ± 75	302–474	385 ± 57

It should be noted that the amounts of silver in the brain of females and offspring was almost the same with or without counting the silver content in blood vessels. Thus, it can be suggested that there exists certain rate of AgNPs' absorption to the brain which does not directly depend on AgNPs's content in the blood.

Conclusions

Neutron activation analysis proved to be an efficient tool to determine the content of silver accumulated in different biological tissues at different ways of AgNPs administration—by oral consumption with liquids (water or milk) and by transfer through placental barrier. Silver was present in all examined organs of experimental females and their offspring. The content of silver determined in experimental animals was significantly higher than in control mice. The mean content of silver in lungs, liver, kidneys and brain of female and offspring was nearly the same, while silver content in offspring's blood was significantly higher. Thus, influence of nanoparticles on females deserves special attention, since nanoparticles can be accumulated in offspring.

Acknowledgements This work was supported by the Russian Foundation for Basic Research (RFBR) [Grant No. 19-015-00145 A].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiments involving mice had been approved by local Ethics Committee and met the requirements of the Directive 2010/63/EU of the European Parliament and of the Council from September 22, 2010 on protection of animals used for scientific purposes and in compliance with the American College of Toxicology Policy on the Use of Animals in Toxicology.

References

1. Yang H, Sun C, Fan Z et al (2012) Effects of gestational age and surface modification on materno-fetal transfer of nanoparticles in murine pregnancy. Sci Rep 2:847
2. Campagnolo L, Massimiani M, Vecchione L et al (2017) Silver nanoparticles inhaled during pregnancy reach and affect the placenta and the foetus. Nanotoxicology. https://doi.org/10.1080/17435390.2017.1343875
3. Takeda K, Suzuki K, Ishihara A et al (2009) Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. J Health Sci 55:95–102
4. Morishita Y, Yoshioka Y, Takimura Y et al (2016) Distribution of silver nanoparticles to breast milk and their biological effects on breast-fed offspring mice. ACS Nano. https://doi.org/10.1021/acsnano.6b01782
5. Recordati C, De Maglie M, Bianchessi S et al (2016) Tissue distribution and acute toxicity of silver after single intravenous administration in mice: nano-specific and size-dependent effects. Part Fibre Toxicol 13:12
6. Sweeney S, Adamcakova-Dodd A, Thorne PS, Assouline JG (2018) Multifunctional nanoparticles for real-time evaluation of toxicity during fetal development. PLoS ONE. https://doi.org/10.1371/journal.pone.0192474
7. Chu M, Wu Q, Yang H et al (2010) Transfer of quantum dots from pregnant mice to pups across the placental barrier. Small 6:670–678
8. Yamashita K, Yoshioka Y, Higashisaka K et al (2011) Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 6:321–328
9. Semmler-Behnke M, Kreyling WG, Lipka J et al (2008) Biodistribution of 1.4- and 18-nm gold particles in rats. Small 4:2108–2111
10. Sharma A, Cornejo C, Mihalic J et al (2018) Physical characterization and in vivo organ distribution of coated iron oxide nanoparticles. Sci Rep 8:4916
11. Sadauskas E, Wallin H, Stoltenberg M et al (2007) Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 4:10
12. Aengenheister L, Dietrich D, Sadeghpour A et al (2018) Gold nanoparticle distribution in advanced in vitro and ex vivo human placental barrier models. J Nanobiotechnol 16:79
13. Lasagna-Reeves C, Gonzalez-Romero D, Barria MA et al (2010) Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. Biochem Biophys Res Commun 393:649–655
14. Perez-Jordan MY, Soldevila J, Salvador A et al (1998) Inductively coupled plasma mass spectrometry analysis of wines. J Anal At Spectrom 13:33–39
15. De Jong WH, Hagens WI, Krystek P et al (2008) Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials 29:1912–1919
16. Frontasieva MV (2011) Neutron activation analysis in the life sciences. PEPAN 42:332–378. https://doi.org/10.1134/S1063779611020043
17. The methodological recommendations for euthanasia of small pet animals. https://www.kostromavet.ru/files/files/Evtanaziy_09_06_14.pdf. Accessed 15 Aug 2019 (**in Russian**)
18. Zinicovscaia I, Pavlov SS, Frontasyeva MV et al (2018) Accumulation of silver nanoparticles in mice tissues studied by neutron activation analysis. J Radioanal Nucl Chem 318:985–989
19. Greenberg RR, Bode P, De Nadai Fernandes EA (2011) Neutron activation analysis: a primary method of measurement. Rev Spectrochim Acta Part B 66:193–241
20. Pavlov SS, Dmitriev AYu, Frontasyeva MV (2016) Automation system for neutron activation analysis at the reactor IBR-2, Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia. J Radioanal Nucl Chem 309:27–38
21. Valentini X, Rugira P, Frau A et al (2019) Hepatic and renal toxicity induced by TiO_2 nanoparticles in rats: a morphological and metabonomic study. J Toxicol 2019:5767012
22. Lee Y, Choi J, Kim P et al (2012) A transfer of silver nanoparticles from pregnant rat to offspring. Toxicol Res 28:139–141
23. Du B, Yu M, Zheng J (2018) Transport and interactions of nanoparticles in the kidneys. Nat Rev Mater 3:358–374
24. Purves D, Augustine GJ, Fitzpatrick D, Katz LC, La Mantia AS, McNamara JO, Williams SM (2001) Neuroscience, 2nd edn. Sinauer Associates, Sunderland
25. Antsiferova AA, Buzulukov YuP, Demin VA et al (2015) Radiotracer methods and neutron activation analysis for the investigation of nanoparticle biokinetics in living organisms. Nanotechnol Russ 10:101–108